

HOLBROOK (M.L.)

Microscopical researches
of the Corpuscular elements
of blood

FROM
PROCEEDINGS

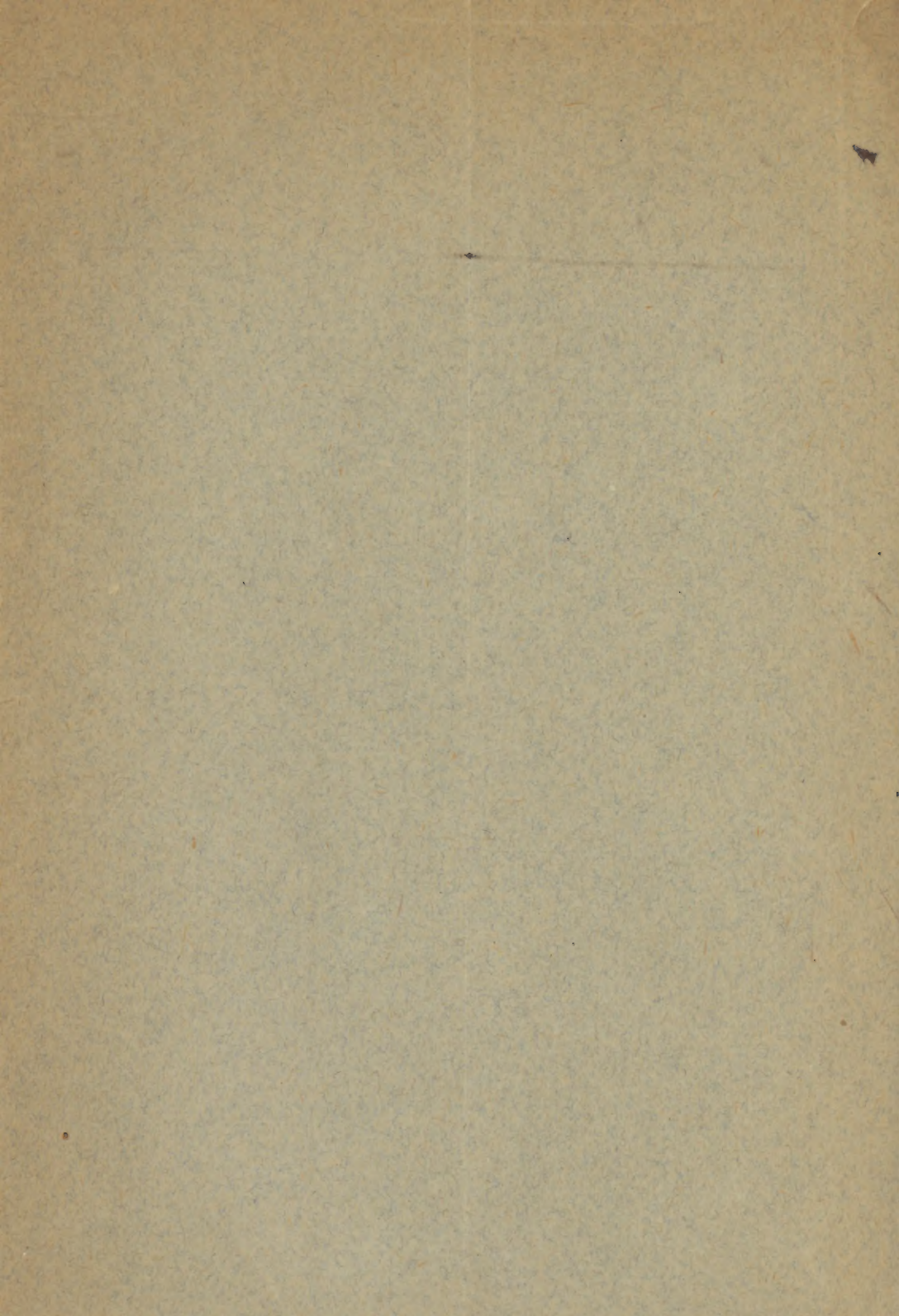
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MICROSCOPICAL RESEARCHES OF THE CORPUSCULAR ELEMENTS OF BLOOD.

M. L. HOLBROOK, M. D., New York City.

I.

The Structure of the Red Blood Corpuscles of Man.

Although since the time of the discovery of the blood corpuscles, over 200 years ago, a good many observations have been made by various microscopists, certain questions still remain unsettled. One of these questions is, Are the red blood corpuscles only structureless formations, of certain chemical compounds, or are they endowed with properties of life? A second question is, What is the structure of the colorless corpuscles of the blood, and in what relation do they stand to the constitution? A third question is, What is the source and significance of the so-called third element of the blood, the blood plaques, to the presence of which attention has recently been called? I shall in this paper consider only the red blood corpuscles, their structure, their varying appearance under the microscope, and their relationship to other protoplasmic formations. The other questions I must postpone to some future occasion, because my studies in them have not been completed.

It seems to me plain that the living organism, being traversed by a closed vascular system, cannot carry only chemical compounds destitute of life, as morphological elements. The liquid portion unquestionably is a carrier of such compounds, holding a good deal of albumen, fibrine, and certain salts; carrying also the nourishing matter destined for nutrition; also effete materials that are destined to be eliminated by the secretory organs. The corpuscles, on the contrary, serve as the carriers of a complex and highly oxidized material, which we call hæmoglobin. Obviously this hæmoglobin, important as it is, is destitute of life; whereas the bearers of it, or



the red blood corpuscles, must be alive and subject to changes in form and in other properties which we find only in living matter.

In our present views of the animal organism we consider the so-called protoplasm as the real seat of life. It has been shown, however, that protoplasm is by no means structureless; that what has previously been termed granules of the protoplasm are really the points of intersection of a minute reticulum traversing it. This reticulum has been shown to be the contractile or living matter proper; whereas in the meshes of this reticulum a liquid is present which, though varying in consistency and chemical composition, is acknowledged to hold nitrogen and to serve as the carrier of both nourishing and effete materials. It has been shown that the animal organism is a continuous mass of protoplasm, continuous throughout all the tissues, the consistency of which depends upon the chemical changes of the liquid, semi-solid, or solid filling material of the meshes; whereas the reticulum of the living matter proper is not only continuous, but also identical throughout all the tissues of the organism, both in animals and in plants.

The main mass of the animal body, aside from its fluid portion, is the so-called connective tissue, which, by a varying consistency of its basis substance, furnishes the organism with shape and firmness. The skeleton of the vertebrates is a variety of connective tissue, mainly destined to build up the frame. Dense and highly elastic formations of connective tissue are represented by the different varieties of cartilage covering the articular ends of the bone, giving firmness to air passages, to the frame of the nose, the orifice of the ear, etc. Highly elastic representatives of fibrous connective tissue are the tendons, ligaments, the fascia, aponeurosis, and the derma of the skin. More delicate varieties of fibrous connective tissue accompany and hold together all the formations of higher grades of vitality, such as the muscle fibers and the nerves. The connective tissues we know to be simply supporters of the blood and lymph vessels. Neither muscle nor nerve fibers do as such contain blood or lymph vessels. It is invariably the accompanying connective tissue that holds such formations; yet the muscle fibers are endowed, so to say, with far more vitality than the connective tissue, since they serve for motor purposes. We know that the living or contractile matter is contained in the muscles in large quantities; that the constituent, primitive, sarcous elements, though considerably larger than the granules of ordinary protoplasm, are identical with the latter, and are interconnected throughout the motor apparatus

by delicate threads. The central formation of the nervous system, the gray substance and its offshoots, the axis cylinders are again reticulated in structure, but far more delicate than in the muscles, evidently fitting them for rapid contractions of the reticulum, conveying sensory and motor impulses with great rapidity through the organism. The epithelial tissue is likewise destitute of blood and lymph vessels, but owing to the presence of the horny filling material of the meshes endowed with a high degree of resistance, such as we observe in the scarf skin, the hairs, the crystalline lens. The epithelia performs all the secretory work of the body through the material carried into them by the accompanying blood vessels located in fibrous connective tissue. The real structure of the epithelia is the same as that of protoplasm in general, although the chemical function is specifically that of secretory and excreting effete material.

In blood we have only a liquid, the so-called serum or plasma, in which there are present large numbers of red and smaller numbers of colorless corpuscles, all of which are single, independent individuals rolling about in the serum, and, as far as the red blood corpuscles are concerned, serving as the carriers of oxidizing and nourishing materials. This liquid, according to our present views, is certainly not entitled to the name of a tissue. In every amæba we occasionally observe vacuoles containing a liquid in which we see detached granules of living matter. The vascular system of the amæba—that is, the vacuoles—is obviously homologous to the closed vascular system of higher animals, the only difference being that the vacuoles are transient, the blood vessels permanent formations. The granules of living matter suspended in vacuoles are probably homologous with the blood corpuscles in the blood of higher animals.

In order to examine fresh blood under the microscope we must keep it in a moist chamber to prevent the evaporation of its fluid parts for some length of time. To secure this we oil the edges of a thin covering glass with any oil, sweet-oil, cod-liver oil, castor-oil, or vaseline; glycerine is unfit, since it is soluble in water. For applying the oil use a little roll of paper in the form of a very small rod. The skin, best of the palm of the left thumb, is pricked with a sharp needle, rendered aseptic by heating it in the flame of a spirit-lamp. The drop of blood should be small; therefore the prick need not be deep. The painful sensation is reduced to a minimum by firm compression of the parts to be pricked with the finger and thumb of the

operator's left hand. A slight alternate pressure or squeezing and relaxing will in a few seconds secure a droplet of blood, the size of which should not exceed that of a medium-sized pin-head. The slide should be applied at once over the droplet. A rotatory movement of the slide serves to spread it evenly, and immediately the cover, which has been prepared in advance and so placed that it may be quickly seized, is put over it. Its oiled edges should hermetically seal in the blood and prevent evaporation. The bleeding from the prick soon ceases, and in order to avoid infection, which would rarely happen, we may apply to the wound a little dilute acetic acid or a weak solution of sublimate, or even hot water.

Some practice is required to get just sufficient oil on the edges of the cover. Too little allows the penetration of air from the outside, thus rendering the specimen unfit for observation. Too much causes a concentration of the blood and an overcrowding of the corpuscles, which prevents their individual observation, and also mixes oil globules with the blood serum. If the specimen be properly prepared it remains fit for study for about fourteen days. Such a protracted study is the special feature of my procedure and the result is my present paper. I have examined specimens of blood from about thirty persons, of varying ages and constitutional characteristics, including both sexes. The youngest was a tuberculous child four years old, the oldest a woman of seventy-six.

The drop of blood after mounting should be at once transferred to the stage of the microscope and studied with a good immersion lens (water or cedar oil) of an amplifying power of at least 1,000 or 1,200. My studies were made in C. Heitzmanh's laboratory, with a $\frac{1}{16}$ -inch immersion lens made by the late Tolles, of Boston, which, as shown by comparison, I have found superior to any other immersion lens, not excluding the celebrated ones by Zeiss, of Jena.

The first thing which strikes the student is the great variety in the tint of the red blood corpuscles. The deepest tint I found in the blood of a Vassar girl, twenty-six years old, whose anthropometric measurements gave the largest respiratory capacity in a class of five hundred girls. Another very brilliant specimen was in a woman seventy years old, of remarkably strong body and vigorous health, and withal an abstainer from flesh food. The palest tint was seen in the blood of an Irish servant girl, about twenty-five years of age, who, although strong and healthy before coming to our shores five years ago, became pale and chlorotic here, as so many of these girls do, on account of their changed and unhygienic

habits of eating, dress, and absence of outdoor active life. All grades between these extremes were observed in other subjects. A peculiar *lake* color was noticed in the red blood corpuscles of a girl, twenty years old, who had just passed an attack of parotitis (mumps). A pronounced dusky lake color was seen in the blood corpuscles of a man, thirty-five years old, whose spleen was noticeably enlarged. A still more pronounced hazy lake color was seen in the red corpuscles of a physician, forty-two years of age, who a few months after the observation died with sarcoma of the lungs; and still another case of a deep lake color was observed in a woman, over fifty years of age, whose kidneys were in a cirrhotic condition.

A second striking feature under the microscope was the variation in the coin-like arrangement of the red blood corpuscles. This was found invariably in the blood of persons of a good constitution, less frequently in the blood of individuals of a midling constitution, and almost absent in persons of a very poor constitution, especially those who had tuberculosis, chlorosis, or who were broken down by acute or chronic ailments.

In one instance observation extending over years showed a temporary presence and absence of these coin-like rolls. It is quite probable that the agglutination of the red blood corpuscles is due to the presence of fibrine in the serum of the blood.

A third striking feature was the appearance of crenations along the borders of the corpuscles immediately after their transference to the microscope, which in most instances required not more than one minute after the prick of the skin. The crenations were most numerous and conspicuous on the red blood corpuscles of persons of an excellent constitution, with a pronounced red color.

It has been said that these crenations are caused by alcoholic drink, but in one specimen from a man with a fine constitution nearly every one was crenated, and I can testify to his being absolutely temperate. Only the smallest sized corpuscles, termed since Hagen, 1876, hæmatoblasts, never become crenated, though in one case I did observe very delicate thorn-like projections from their margin. This was in the case of a broken-down man, about sixty years old, with evidence of anæmia, in which the hæmatoblasts existed in an abnormal number. I estimated that they amounted to about one-fourth of the total number of corpuscles.

The chlorotic girl before mentioned had no crenated red blood corpuscles upon immediate examination, but three to four hours later a limited number were slightly crenated. After forty-eight

hours there were still more. A very large number showed odd and unusual forms. The same girl, after a month at the sea-side, had far less unusual forms of corpuscles and one-third or more were crenated soon after being put on the slide. I have found, as a general rule, that the deeper the red color of the corpuscles or, in other words, the more highly oxidized the hæmoglobin the sooner the crenations appear and the more numerous they are. On the contrary, the paler the red color of the corpuscles the later will the crenations take place and the scantier and more irregular they are. The fewest I have seen was in the red blood corpuscles of the tuberculous four-year-old child quoted above.

The crenations after several days, usually four to six days, change their character. Instead of showing knobbed or globular protuberances, they become more delicate and thread-like. On the sixth and eighth day they are mostly gone, except in these cases where they appear late, and their contours are, in the majority of corpuscles, even and regular. The knob and thread-like prolongations along the periphery of the corpuscles appear in the shape of delicate globules of a somewhat higher refraction on the surface of the disk being looked at from a top view. I emphasize this feature since it has misled some observers. For instance, Hueter, of Germany, took the knobs on the surface of the disk for micro-organisms held in their interior. I would discard every blood corpuscle with serrated edges for the study of its interior structure, since this is rendered deceptive by the presence of knobs and folds or wrinkles which resemble the reticulum and have misled some observers, as already stated.

The next question arises, What causes the crenation of the red blood corpuscles? Elsberg was the discoverer of the reticular structure of these formations.*

His observations were made on blood treated with a solution of bichromate of potash in distilled water, diluted so as to make about a five per cent. solution. He saw shortly after the application of the preparation varying changes of the corpuscles by indentations and by protrusion, aside from vacuation of the flattened-out disks, and came to the conclusion that these changes of form were due to the contraction of the reticulum prevading the body of the corpuscles. Such a contraction of a regularly arranged reticulum causes

*The structure of the colored blood corpuscles. *Annals of New York Academy of Sciences*, 1879.

bulging of the portion of the wall of the corpuscle between the net corresponding to a mesh filled with hæmoglobin. Elsberg maintained that this reticulum is living or contractile matter proper, the same as in any other protoplasmic body. The meshes, on the contrary, instead of holding a nitrogenous liquid, such as in ordinary protoplasm, holds the chemical compound termed hæmoglobin. All that is done by the solution of bichromate of potash is the extraction of the hæmoglobin from the meshes of the reticulum, whereby this latter is rendered plainly visible. According to Elsberg, therefore, a red blood corpuscle is nothing but a lump of protoplasm holding in its meshes a liquid substance termed hæmoglobin, otherwise endowed with all the properties of life which are generally attributable to protoplasm.

Elsberg's great discovery has met with incredulity and unjust criticism. Weichselbaum, of Vienna, objects to the value of the discovery on the ground that the solution of bichromate of potash has altered and mutilated the structure of the corpuscle, though he ought to have known that this substance and also chromic acid in weak solution is admitted by all histologists to be the best preservative of tissues—in fact, directly proven under the microscope some thirty years ago by Alexander Rollett. Mueller's preserving fluid is composed of one per cent. of bichromate of potash and two per cent. of sulphate of soda, and is still largely in use for the preservation of the most delicate tissues, such as the brain, the spinal cord, the eye-ball, especially the retina. L. Ranvier, of Paris, who is considered the best French histologist, could not satisfy himself as to the presence of a reticulum in the interior of the red blood corpuscles, but considers Elsberg's figures as foldings on the surface of the corpuscles. Only quite recently Dr. Alfred C. Stokes, of Trenton, New Jersey,* has, however, enthusiastically and in strong language confirmed Elsberg's observations point by point. I am also able to confirm them by a method which I hope is beyond question, and this method is to simply observe the same mounted droplet of blood up to the eighth or tenth day, prepared as previously described. By closely watching the red blood corpuscles we notice a gradual fading of these bodies, with a simultaneous gradual coloring of the serum in which the corpuscles are suspended. Since no reagent was used in my specimens, the conclusion I draw is that the serum gradually dissolves the hæmoglobin from the corpuscles, thus

*The Microscope, vol. 12, No. 2.

rendering its interior structure visible. Even corpuscles of a deep color will, a few days after mounting, become colorless or nearly so, and the reticulum in their interior will come plainly into view, the meshes being light after the extraction of the hæmoglobin, and the reticulum gray or greenish-gray. This observation is the more reliable, as, at the period of time mentioned, all crenations and folds on the surface disappear. Comparison of corpuscles treated according to the method of Elsberg with my own method compels me to say that the reticulum may be a trifle more conspicuous or regular in specimens treated with the solution of potash than in specimens not treated at all, which is my own method; but there is not the slightest difficulty to a trained eye armed with a good immersion lens to see the reticulum or structure in untouched specimens, nor of convincing one's self of its identity with that of Elsberg.

Beginning from the twelfth day and much earlier in blood from sick patients, especially with tuberculosis, the corpuscles begin to break asunder into granules, the reticular connections of which still remain visible in many cases.

At the same time other corpuscles may become so faded that only faint, ghost-like shadows remain. About the fourteenth day after mounting the blood the specimen is, owing to these changes, rendered unfit for further observation, the serum having by this time assumed a reddish-yellow tint, owing to the hæmoglobin being diffused through it.

In the meantime other remarkable changes have taken place, which throw light upon the origin and significance of the so-called third element of the blood. The description of these occurrences I defer to some future time.

